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Short Communication

Human platelets antigens influence the viral load of platelets after the interaction of the platelets with HCV and HIV *in vitro*

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Abstract

Introduction: In this study, we evaluated hepatitis C virus (HCV) and human immunodeficiency virus (HIV) – platelet interactions *in vitro* as well as human platelets antigen (HPA) polymorphisms. **Methods:** Platelets were obtained from 100 healthy HPA-genotyped volunteer donors and incubated with HIV or HCV. The viral load after *in vitro* exposure was detected. **Results:** The viral load in the platelets after exposure to the virus was higher in the HIV exposure than in the HCV exposure. **Conclusions:** HIV-platelet ligation could be more efficient than HCV-platelet interaction. Further, the HPA-1b allele seems to influence the interaction of platelets with HCV.

Keywords: Hepatitis C virus. Human platelets antigen polymorphisms. Human immunodeficiency virus.

Platelets have been considered a carrier of the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in the blood circulation of infected patients[1] [2]. However, platelets do not express cluster of differentiation 4 (CD4) and cluster of differentiation 81 (CD81) molecules, which are the main receptors associated with HIV and HCV interactions, respectively, in their target cells[3] [4], indicating that other molecules might play a role in the interaction of HIV and HCV with platelets.

Many viruses use molecules involved in cell adhesion as receptors or co-receptors for example, rhinovirus uses intercellular adhesion molecule 1 (ICAM-1)[5] and adenoviruses use integrins[6]. Moreover, platelets express proteins of the integrins family, such as glycoprotein (GP) complex GPIa-IIa and GPIIb-IIIa, which contain polymorphic antigenic determinants called human platelet antigens (HPA)[7]. The HPA-1, HPA-3, HPA-4, and HPA-5 systems have been the most studied ones in platelet disorders[8]. HPA polymorphism has already been shown to be associated with viral infections such as dengue[9]. Platelets are known to interact with both HIV and HCV *in vitro*[10] [11], but it is still unknown if the interaction with HCV and HIV occurs in the same way or if it depends on some viral or genetic factors.

In this context, the aim of this study was to evaluate the differential viral load in platelets after HCV and HIV platelet interactions *in vitro* and to determine if HPA polymorphisms could modify these interactions.

Platelets were obtained from peripheral venous blood from 100 healthy HPA-genotyped volunteer donors at the Blood Transfusion Center, Botucatu Medical School, Sao Paulo State University (UNESP) SP, Brazil. The inclusion criteria were absence of ribonucleic acid-human immunodeficiency virus (RNA-HIV) and ribonucleic acid- hepatitis C virus (RNA-HCV) confirmed by molecular assays and signed informed consent. Fifty of these volunteers were used in the HCV experiment and all 100 of them were used for the HIV experiment.

Deoxyribonucleic acid (DNA) was isolated from the total blood of all 100 donors and was used to genotype HPA-1 and -3 by polymerase chain reaction-sequence specific primer (PCR-SSP) as described by Klüter et al.[12] and HPA-5 was genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described by Kalb et al.[13] The platelet pellets were obtained using the protocol described by Padovani et al.[14]

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The genotyped platelet pellets were separately incubated with 1mL of plasma pool containing 100,000UI/mL^{-1} subtype B HIV or genotype 1 HCV virus, respectively, in a micro tube, for 48 hours at 37°C. The viral load (for HCV or HIV) after these *in vitro* exposures was detected using quantitative-PCR with a lower detection limit of 1.70 log using Abbott real-time PCR system (Abbott Molecular Inc., Des Plaines, IL).

The analyses were performed based on the viral load – (G1) RNA lower or equal 1.70 log or (G2) RNA upper 1.70 log – in order to quantitatively evaluate the influence of HPA polymorphisms on the interaction of HCV or HIV with the platelets.

All the procedures were performed using negative controls to guarantee the veracity of the results. The χ^2 test was used to determine possible differences in the platelet viral load after *in vitro* HCV- or HIV-platelet interactions. The Hardy-Weinberg equilibrium test was performed to evaluate the distribution of allelic frequencies of HPA -1, -3, and -5 among the groups. The χ^2 test was used to identify possible associations of the RNA viral load in the platelet with the genotype frequencies and allelic frequencies of HPA. The significance level for all the statistical tests was set at 0.05.

Viral load in the platelets obtained from the donors exposed to HCV was lower than that in the platelets obtained from the donors exposed to HIV. No platelets exposed to the HIV presented an undetectable viral load (lower 1.70 log) after viral exposure. On the other hand, platelets from 21 (42%) donors exposed to HCV presented an undetectable viral load (lower 1.70 log) after viral exposure.

There were no significant differences in the allelic and genotypic frequencies for any of the evaluated HPA systems when the HIV RNA loads in the platelets were considered [p = 0.460, 0.172, and 0.780 for the genotypic frequencies of HPA-1, -3, and -5, respectively; p = 0.575, 0.10, and 0.79 for the allelic frequencies of HPA -1, -3, and -5, respectively (data not shown)].

Table 1 shows the relation between the viral load of platelets exposed to HCV and the allelic and genotypic frequencies of HPA-1, -3, and -5. There were no significant differences in the genotypic frequencies for any of the HPA systems when the undetectable (lower 1.70 log) and detectable (upper 1.70 log) HCV RNA loads in the platelets were considered (p = 0.0676, 0.5183, and 0.7683 for HPA-1, -3, and -5, respectively). However, the HPA-1 system deviated from the Hardy-Weinberg equilibrium: the frequency of the HPA-1b allele was significantly reduced (p = 0.0272) in the platelets with detectable HCV RNA loads.

In this study, we showed that although both HCV and HIV interact with platelets *in vitro*, the RNA viral load in platelets after viral exposure is higher when the platelets are exposed to HIV than when they are exposed to HCV, which suggests that HIV-platelet ligation could be more efficient than HCV-platelet interaction. To the best of our knowledge, this is the first report of its kind to date.

It has already been demonstrated that platelets interact with HIV and HCV *in vitro*[10] [11], although they do not express the entry receptors for these viruses[3] [4], which could be due to the presence of other molecules in platelet membranes, such as CXCR4, DC-SIGN, and CLEC-2, which are already know to be associated with the capture of HIV by platelets [3] [15]. Consistent with this, our findings suggest that HPA -1, -3, and -5 polymorphisms do not interfere with HIV-platelet interaction *in vitro*, but platelet-HCV interaction seems to be influenced by the presence of the HPA-1b allele. This result suggests that the HPA-1b allele could be, in some way, associated with lower viral loads, which could be advantageous *in vivo*. In the future, further studies should be conducted to elucidate the mechanism of the interaction of HCV with platelets and to elucidate the real role of the HPA-1b allele in this interaction, since there are still controversies with regard to whether platelets could act as a reservoir for HCV during infection.

TABLE 1

Allelic and genotypic frequencies for HPA-1, -3, and -5 according HCV load in platelets from uninfected individuals exposed to the virus (n = 50).

	Undetectable (lower 1.70 log)		Detectable (upper 1.70 log)		
HCV Load	**n**	**%**	**n**	**%**	***p-value***
HPA alleles					
HPA-1a	25	30.9*	56	69.1*	0.02
HPA-1b	11	57.9*	8	42.1*	
HPA-3a	27	38.0	44	62.0	0.50
HPA-3b	9	31.0	20	69.0	
HPA-5a	32	35.5	58	64.5	0.78
HPA-5b	4	40.0	6	60.0	
HPA genotypes					
HPA-1a/1a	9	27.3	24	72.7	0.06
HPA-1a/1b	7	46.7	8	53.3	
HPA-1b/1b	2	100.0	0	0.0	
HPA-3a/3a	12	41.4	17	58.6	0.51
HPA-3a/3b	3	23.1	10	76.9	
HPA-3b/3b	3	37.5	5	62.5	
HPA-5a/5a	14	35.0	26	65.0	0.76
HPA-5a/5b	4	40.0	6	60.0	
HPA-5b/5b	0	0.0	0	0.0	

HPA: human platelet antigen. **HCV:** hepatitis C virus. *significant difference.

Ethical considerations

This study was approved by the Research Ethics Committee of Botucatu School of Medicine, Sao Paulo State University (UNESP).

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Conflict of Interest

The authors declare that there is no conflict of interest.

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